



Methane Mitigation Strategies by Optimizing Nutrient Profiles in an Eco-Friendly Mixture of Cassava Pulp and *Indigofera Zollingeriana* Branch Silage with Strategic Protein Supplementation

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Abstract | Enteric methane emissions from ruminants are reportedly contributing to climate change, a problem intensified by livestock feeding practices. Therefore, this study aimed to evaluate alternative protein sources that could improve nutrient utilization, reduce methane emissions, and enhance feed efficiency in ruminant diets for mitigated environmental impacts. The effects of different protein sources were assessed on nutrient content, *in vitro* rumen fermentation characteristics, gas production, methane emissions, and degradability of a basal feed mixture consisting of Cassava pulp (C) and *Indigofera zollingeriana* branch (I) silage. The basal feed silage (CI) was supplemented with protein sources including *Indigofera zollingeriana* leaf meal (CIIZM), jack bean meal (CIJBM), palm kernel meal (CIPKM), soybean meal (CISBM), and black soldier fly larvae meal (CIBSM). The results showed that gas production was highest in CISBM (159.40 ml/g DM) and CIBSM (159.17 ml/g DM), but lowest in CI (141.92 ml/g DM). Methane production, as a proportion of total short-chain fatty acids (TSCFA), was significantly lower in CISBM (11.33%), CIBSM (11.59%), and CIIZM (12.02%) compared to CI (20.78%), suggesting that these feed combinations may effectively mitigate methane emissions. Degradability was also highest in CISBM (65.79% IVDMD) and CIBSM (65.87% IVDMD). Based on the results, incorporating black soldier fly larvae meal and *Indigofera zollingeriana* leaf meal into ruminant diets can enhance fermentation efficiency, reduce methane emissions, and improve degradability. This offers a promising strategy for reducing reliance on conventional protein sources such as soybean meal, thereby contributing to more sustainable livestock feeding practices.

Keywords | Cassava pulp, Eco-friendly, *Indigofera*, Methane, Mitigating, Silage

Received | October 05, 2024; **Accepted** | November 13, 2024; **Published** | December 31, 2024

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Citation | Ridla M, Nahrowi (2025). Methane mitigation strategies by optimizing nutrient profiles in an eco-friendly mixture of cassava pulp and *Indigofera zollingeriana* branch silage with strategic protein supplementation. Adv. Anim. Vet. Sci. 13(1): 198-208.

DOI | <https://dx.doi.org/10.17582/journal.aavs/2025/13.1.198.208>

ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331



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INTRODUCTION

The global food system is facing a dual challenge namely providing sufficient protein for a growing population

and mitigating the environmental impact of livestock production (Safdar *et al.*, 2023). Livestock practices that rely heavily on resource-intensive feed sources, such as soybean meal, significantly contribute to deforestation, biodiversity

loss, and greenhouse gas emissions. Enteric methane emissions from ruminants, which arise from microbial fermentation in the rumen, are a particularly critical contributor to climate change, accounting for approximately 44% of total agricultural methane emissions globally (Tseten *et al.*, 2022; Palangi *et al.*, 2022). The inefficiencies of current livestock feeding practices intensify this problem, underscoring the need for more sustainable and eco-friendly feed alternatives.

Although feed additives and dietary manipulation (Haque, 2018; Haryati *et al.*, 2019) have shown some promise, these methane mitigation strategies are costly and inconsistent, often requiring large inputs of resource-intensive ingredients, such as soy (Hristov *et al.*, 2022). Reliance on conventional protein sources adds to the environmental burden and limits the scalability of methane-reducing strategies, particularly in regions with limited agricultural resources. Addressing these challenges requires exploring alternative feed ingredients that are both cost-effective and environmentally sustainable (Batbekh *et al.*, 2023).

Cassava pulp, a byproduct of the *Manihot esculenta* starch industry, is gaining recognition as a potential solution to this problem (Sengxayalth and Preston, 2017). With the high fiber and starch content, Cassava pulp is an energy-dense, low-cost feed option that supports microbial fermentation in the rumen. However, the low protein content necessitates supplementation with protein-rich additives to achieve a balanced diet (Keaokliang *et al.*, 2018). Previous studies by Norrapoke *et al.* (2022) have shown that Cassava pulp when combined with protein-rich ingredients such as soybean meal, can enhance nutrient utilization and support livestock growth. However, the potential for methane reduction remains less explored. The reliance on soy also raises concerns due to the environmental impact.

Indigofera zollingeriana, a tropical legume, offers a sustainable alternative to traditional protein sources. The study shows that incorporating *Indigofera zollingeriana* meal, a protein-rich tropical legume, may enhance rumen fermentation efficiency and animal performance, supporting eco-friendly livestock systems (Antari *et al.*, 2022). The ability to grow in less fertile soils and low-input conditions underscores the role as a sustainable protein source, capable of reducing reliance on environmentally harmful feed components (Herdiawan, 2016). Furthermore, the branch, though lower in protein than the leaves, contributes to fiber intake and supports gut health when included in silage mixture.

Preserving Cassava pulp through ensiling stabilizes nutritional value and extends shelf life, making it a practical and sustainable feed option for ruminants (Taysayavong *et al.*, 2018). Combined with *Indigofera zollingeriana* branch, the resulting silage offers a balanced blend of energy and

protein, with moderate fiber content that enhances rumen health and digestion. This combination, as shown by Ridla *et al.* (2023a), can potentially optimize rumen fermentation, reduce methane production, and improve livestock performance.

Although Cassava pulp and *Indigofera zollingeriana* are beneficial for ruminant nutrition, the combined effects on methane emissions, nutrient degradability, and fermentation efficiency remain underexplored. Most existing studies were centered on traditional feed ingredients such as soybean meal, neglecting the potential of less resource-intensive alternatives to achieve similar or improved outcomes. This study was conducted to address the current gap by evaluating the impact of Cassava pulp and *Indigofera zollingeriana* branch silage, combined with various protein sources, on rumen fermentation, methane emissions, and degradability *in vitro*. The objective was to identify whether integrating eco-friendly ingredients into ruminant diets can offer a viable alternative to soybean meal, reducing methane emissions and enhancing fermentation efficiency, thereby promoting environmental sustainability.

MATERIALS AND METHODS

SILAGE AND DIET FORMULATION

This study developed treatment mixture incorporating various feed components, including cassava pulp (C), *Indigofera zollingeriana* branch (I), *Indigofera zollingeriana* leaf meal (IZM), jack bean meal (JBM), palm kernel meal (PKM), soybean meal (SBM), and black soldier fly larvae meal (BSM). A total of six distinct treatment mixture of Cassava pulp and *Indigofera zollingeriana* branch silage were prepared on a dry matter (DM) basis, as follows: CI (50% C + 50% I) silage as the basal feed, CIIZM (70% basal feed + 30% IZM), CIJBM (70% basal feed + 30% JBM), CIPKM (70% basal feed + 30% PKM), CISBM (70% basal feed + 30% SBM), and CIBSM (70% basal feed + 30% BSM).

These proportions, informed by previous studies and empirical observations, were designed to achieve the necessary nutritional balance to support optimal rumen microbial fermentation while addressing environmental concerns such as methane emissions (Ridla *et al.*, 2023a; Ridla *et al.*, 2023b; Mulianda *et al.*, 2020).

SILAGE PRODUCTION

Silage production followed the method of Ridla and Uchida (1998), using six replicates of the mixed CI silage samples packed into 2-liter high-density polyethylene (HDPE) bottles. Samples were compacted to remove air, then sealed and stored at room temperature for 40 days. After the ensiling period, silos were reweighed to determine fermentation losses, including DM loss due to gas production.

Post-production quality assessment was carried out by dividing approximately 500 g of each CI silage into two portions. One portion was diluted with distilled water (1:10) for silage fermentation measurements, while the other was oven-dried and ground for further analysis.

SILAGE QUALITY ASSESSMENT

Silage pH was measured using an electric pH meter (Horiba F-12). The ammonia nitrogen (N-NH₃) content, including ammonia in rumen fluid, was determined using the micro-diffusion technique of Conway and O'Malley (1942), while lactic acid concentration was quantified according to the method of Barker and Summerson (1941). Silage quality was assessed using the Flieg point, calculated per Kilic (1986), which combines pH and DM content to provide a comprehensive measure of silage stability and quality.

NUTRIENT CONTENT ANALYSIS

Nutrient analysis followed the Association of Official Agricultural Chemists (AOAC, 2011) protocols to ensure consistency and reproducibility. Moisture content was determined by oven-drying samples at 105°C for 24 hours, crude ash by incineration at 600°C for 4 hours, crude protein (CP) using the Kjeldahl method, crude fiber by boiling samples in acid and base solutions, and crude fat by the ether extraction (EE) procedure. Fiber analysis, specifically for Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF), adhered to AOAC (1990) methods. Water-soluble carbohydrate (WSC), including sugar content, was determined using the anthrone method (Deriaz, 1961), and starch content was measured by acid hydrolysis, as outlined by Pirt and Whelan (1951).

IN VITRO RUMEN FERMENTATION

Rumen fluid was collected from slaughtered animals approximately 15 minutes post-mortem at the IPB University slaughterhouse using thermos bottles pre-filled with water heated to 40°C to preserve integrity. The collection followed the animal care guidelines of Government Regulation No. 41/2014 and Law No. 39/2021 of Indonesia.

In vitro fermentation characteristics and degradability of the diets were assessed by incubating 0.5 g of each feed sample with buffered rumen fluid at 39°C for 48 hours, followed by a secondary 48-hour incubation in an acid-pepsin solution, according to Tilley and Terry (1963). The residue was dried to determine *in vitro* dry matter degradability (IVDMD) and ashed to determine organic matter degradability (IVOMD).

Samples incubated for 4 hours were further analyzed to assess pH using a digital pH meter (Horiba-18). Ammonia nitrogen (N-NH₃) was assessed by using the same method used in the analysis of ammonia for silage quality. Total

short-chain fatty acids (TSCFA) were quantified by steam distillation as described by Kromann *et al.* (1967).

Table 1: Fermentation quality of CI Silage.

Variable	Value
Dry matter, %	38.45 ± 1.22
Dry matter recovery, %	96.34 ± 2.31
pH	4.14 ± 0.01
Lactic acid, % DM	1.67 ± 0.33
Ammonia, % Total N	3.45 ± 0.04
Residual WSC, %	3.23 ± 0.12

WSC: Water soluble carbohydrate.

Gas production, including methane, was measured using the procedure outlined by Theodorou *et al.* (1994). Feed samples (0.75 g) were incubated with rumen fluid and buffered solution in gas-tight vessels at 39°C for 24 hours. Gas production was monitored using a pressure transducer, and methane concentration was quantified through gas chromatography, allowing for a comprehensive analysis of fermentation capacity and methane emissions.

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

To evaluate the effects of varying nutrient compositions in forages, including crude ash, CP, crude fat, crude fiber, non-fiber carbohydrates, NDF, ADF, total sugars, starch, lactic acid, and WSC content, a completely randomized design (CRD) was implemented. This design included six dietary treatments, each with six replicates, to ensure sufficient representation and reduce experimental variability. For the *in vitro* rumen fermentation study, a similar CRD structure was used, also with six dietary treatments and replicates per treatment. Rumen fluid was used to account for biological variability, and randomization was applied to minimize potential bias, with no blocking due to assumed homogeneity among replicates.

Data analysis was conducted using SPSS software version 26, (IBM Corp, 2019). When the primary analysis identified statistically significant differences ($P < 0.05$), post-hoc comparisons were performed using Tukey's Honestly Significant Difference (HSD) test to assess pairwise differences between treatment means. Outliers were detected with Grubbs' test, while the assumptions of normality and homogeneity of variance were verified using the Shapiro-Wilk and Levene's tests, respectively, to ensure the validity and robustness of the statistical model.

RESULTS AND DISCUSSION

SILAGE FERMENTATION QUALITY

Silage sample showed good preservation quality, with key parameters falling within established benchmarks from prior studies (Table 1). The DM content of 28.45% ensured

substantial feed availability, falling within the optimal range of 28–35% reported by Wróbel *et al.* (2023), which is essential for proper fermentation and feed stability. A high DM recovery of 96.34% showed minimal losses during the ensiling process, reflecting efficient preservation, as values above 95% are considered favorable (Borreani *et al.*, 2018).

The pH of 4.14 suggested effective lactic acid fermentation, reaching the critical threshold necessary to inhibit spoilage organisms and maintain silage stability. Optimal pH levels in high-quality silage are generally below 4.2 (Kung *et al.*, 2018). The observed pH level is consistent with a lactic acid content of 1.97% DM, supporting silage stability. According to Okoye *et al.* (2023), lactic acid concentrations in high-quality silage typically range from 2% to 4% of the total DM and can be higher in silages with low DM content.

The ammonia content accounted for 3.45% of total nitrogen, which falls within the recommended range for low protein degradation, showing effective nitrogen preservation. The NH₃-N content in silage is typically less than 10–15% of total nitrogen. High-moisture silage often has higher concentrations of soluble N and NH₃-N compared to drier ones due to more robust fermentation (Kung *et al.*, 2018).

The residual WSC content of 3.23% showed that sufficient fermentable sugars remained post-ensiling, contributing to

palatability and providing a readily available energy source for ruminants. Previous studies (Borreani *et al.*, 2018) suggested that WSC values between 3% and 5% are ideal for maintaining silage quality.

Although P-values were reported for nutrient content, additional statistical analyses further substantiate these results. An analysis of variance (ANOVA) confirmed significant differences ($P < 0.05$) in fermentation quality parameters, with confidence intervals (CI = 95%) providing additional statistical support. These results showed that silage was suitable for ruminant diets, particularly for dairy and beef cattle, where efficient fermentation and nutrient preservation are critical for optimal performance (Kung *et al.*, 2018).

NUTRIENT COMPOSITION OF THE VARIOUS MIXTURE DIETS

Tables 2 and 3 show significant differences in nutrient composition among the six mixtures, indicating varied nutritional profiles and effects on digestion and fermentation efficiency.

CP levels varied significantly ($P < 0.05$) among mixture, with the basal feed (CI) showing the lowest content at 8.82%. In contrast, diets supplemented with protein sources raised CP levels to between 14.54% and 18.72%. Increasing the content is essential for improving the availability

Table 2: Nutrient content of CI Silage and mixture diets.

Variable	CI Silage	CIIZM	CIJBM	CIPKM	CISBM	CIBSM	SEM	P-value
DM, %	89.46	89.19	88.81	88.99	88.96	89.48	0.27	0.242
CA, % DM	3.36 ^b	4.33 ^a	3.11 ^b	4.19 ^a	4.37 ^a	4.35 ^a	0.11	0.046
CP, % DM	8.82 ^d	16.37 ^b	17.79 ^a	14.54 ^c	18.51 ^a	18.72 ^a	0.19	0.015
CF, % DM	27.72 ^a	21.43 ^c	20.44 ^c	24.75 ^b	20.96 ^c	20.02 ^c	0.29	0.034
EE, % DM	1.45 ^d	4.46 ^d	4.12 ^b	4.52 ^b	3.01 ^c	8.53 ^a	0.01	0.038
NFC, % DM	58.54 ^a	54.61 ^b	54.35 ^b	51.71 ^c	54.13 ^b	48.38 ^d	1.79	0.041

DM: Dry matter; **CA:** Crude ash; **CP:** Crude protein; **EE:** Ether extract; **CF:** Crude fiber; **NFC:** Non-fiber carbohydrate; **CI** (50% Cassava pulp + 50% *Indigofera zollingeriana* branch) silage as the basal feed; **CIIZM:** 70% basal feed + 30% IZM; **CIJBM:** 70% basal feed + 30% JBM; **CIPKM:** 70% basal feed + 30% PKM; **CISBM:** 70% basal feed + 30% SBM; **CIBSM:** 70% basal feed + 30% BSM; **SEM:** Standard error of the mean; ^{a-d}Means in the same row without a common letter are different at $P < 0.05$.

Table 3: Structural and non-structural carbohydrate components of CI Silage and mixture diets.

Variable	CI Silage	CIIZM	CIJBM	CIPKM	CISBM	CIBSM	SEM	P-value
NDF, % DM	66.69 ^a	56.91 ^c	61.86 ^b	57.09 ^c	55.09 ^c	61.73 ^b	0.27	0.045
ADF, % DM	42.18 ^a	40.90 ^b	38.48 ^c	40.74 ^b	37.34 ^d	37.64 ^d	0.32	0.027
Total sugar, % DM	3.52 ^b	4.19 ^a	3.92 ^b	2.78 ^c	2.91 ^c	2.17 ^c	0.28	0.042
Starch, % DM	5.77 ^a	4.83 ^b	4.62 ^b	4.63 ^b	4.77 ^b	3.82 ^c	0.26	0.033

NDF: Neutral Detergent Fiber; **ADF:** Acid Detergent Fiber; **CI** (50% Cassava pulp + 50% *Indigofera zollingeriana* branch) silage as the basal feed; **CIIZM:** 70% basal feed + 30% IZM; **CIJBM:** 70% basal feed + 30% JBM; **CIPKM:** 70% basal feed + 30% PKM; **CISBM:** 70% basal feed + 30% SBM; **CIBSM:** 70% basal feed + 30% BSM; **SEM:** Standard error of the mean; ^{a-d}Means in the same row without a common letter are different at $P < 0.05$.

of essential amino acids, enhancing microbial protein synthesis, and promoting overall ruminant performance. However, excessive CP can lead to inefficiencies, such as increased nitrogen excretion, which poses environmental concerns (Phesatcha *et al.*, 2021).

The crude fiber (CF) content showed considerable variation ($P < 0.05$), ranging from 20.02% to 27.72%. The basal feed had the highest CF content, while the black soldier fly larvae meal (CIBSM) mixture contained the lowest. Lowering CF levels can enhance nutrient degradability and fermentation efficiency, as reduced CF is associated with faster fermentation rates in the rumen, improving nutrient absorption and microbial activity. However, fiber is crucial for stimulating rumen motility and maintaining gut health, necessitating a careful balance (Cronin *et al.*, 2021).

The results showed significant variation in EE percentages across diets, ranging from 1.45% to 8.53% DM ($P < 0.05$). Higher EE levels enhance energy availability and productivity but excessive dietary fat may negatively impact fiber digestion and rumen health, underscoring the need for careful management. Based on the National Academies of Sciences, Engineering, and Medicine (2021), the recommended total fat concentration (EE) is between 5–6% in the overall diet of ruminants, regardless of the purpose.

The structural carbohydrates including NDF and ADF varied significantly ($P < 0.05$) between diets. The basal feed contained higher NDF and ADF levels (66.69% and 42.18%, respectively), showing greater fiber content, which can slow fermentation and nutrient degradation. Conversely, lower ADF levels in CIBSM at 37.34% DM and reduced NDF in CIBSM at 55.09 % DM, suggest improved fermentation potential and higher degradability, leading to better energy utilization by ruminants. The differences between NDF and ADF are crucial for understanding forage quality and the impact on animal feeding behavior. NDF includes more digestible fibrous components and correlates with voluntary intake and rumen fill, showing how much and quickly an animal can consume the feed. In contrast, ADF measures the least digestible components of plant materials, primarily cellulose, lignin, and silica, which significantly influence digestibility and energy availability for ruminants (Carrillo-Díaz, 2022).

Non-structural carbohydrates, including sugars and starch, showed significant ($P < 0.05$) variation, influencing fermentation kinetics. Among the mixtures, CIIZM showed the highest sugar content at 4.19%, contrasting with the lower sugar level in CIBSM at 2.17%. Higher sugar levels promote rapid microbial fermentation, increasing propionate production, which reduces methane emissions and enhances energy efficiency (Beauchemin *et al.*, 2022). However, excessive sugars can lead to rapid fermentation,

risking acidosis (Dong *et al.*, 2021). Starch content was highest in CI (5.77%) and lowest in the CIBSM mixture (3.82%), further supporting rapid fermentation and energy availability but necessitating careful management to prevent fermentation disorders (Villalba *et al.*, 2021).

Achieving a balance between structural and non-structural carbohydrate types is crucial for optimizing rumen fermentation, reducing methane emissions, and improving overall animal performance. Structural carbohydrates (NDF, ADF) ferment slowly, producing acetate and butyrate, which can contribute to higher methane emissions due to hydrogen production. In contrast, non-structural carbohydrates, such as sugars and starches, ferment quickly, promoting propionate production that competes with methanogenesis for hydrogen, thereby reducing methane emissions (Ridla *et al.*, 2023b). This balance is essential for efficient rumen function, as diets that optimize both carbohydrate types can enhance microbial activity, improve nutrient absorption, and minimize environmental impact (Sun *et al.*, 2022).

The results suggest that diets lower in structural carbohydrates and higher in non-structural carbohydrates, such as those containing *Indigofera zollingeriana* leaf meal and black soldier fly larvae meal, promote better rumen fermentation, enhance degradability, and reduce methane emissions. These insights are relevant for improving production efficiency in ruminants, particularly in dairy and beef cattle, where optimized digestion is critical for growth rates and milk yield.

RUMEN FERMENTATION CHARACTERISTICS

Table 4 shows the rumen fermentation characteristics, including pH, ammonia content, and TSCFA, for the basal silage (CI) and various mixture diets.

Although rumen pH showed variation ($P < 0.05$), values remained stable across all diets, ranging from 6.73 to 6.90, indicating a well-maintained environment conducive to efficient microbial fermentation. This pH range falls within the optimal window (5.5 to 7.0) for promoting cellulolytic and amylolytic microbial activity (Öztürk and Gur, 2021). Maintaining rumen pH within this range is essential, as deviations may disrupt microbial populations, particularly fiber-degrading bacteria, which are sensitive to acidic conditions ($pH < 6.2$). When pH falls below this threshold, lactate-producing bacteria proliferate, leading to ruminal acidosis, impairing fiber digestion, and reducing feed efficiency (Weimer, 2022). Conversely, excessively high pH may reduce the activity of starch-degrading microbes, diminishing the production of propionate, a volatile fatty acid (VFA) crucial for energy metabolism (Kitkas *et al.*, 2022). The stable pH observed suggests an optimal balance of microbial

Table 4: Rumen fermentation characteristics of CI Silage and mixture diets.

Variable	CI Silage	CIIZM	CIJBM	CIPKM	CISBM	CIBSM	SEM	P-value
pH	6.90	6.78	6.75	6.88	6.87	6.73	0.01	0.341
NH ₃ , mM	7.05 ^c	9.48 ^b	11.96 ^a	10.23 ^b	12.35 ^a	12.35 ^a	0.18	0.023
TSCFA, mM	71.74 ^c	107.04 ^{bc}	111.86 ^a	91.53 ^b	115.87 ^b	131.68 ^a	2.65	0.011

NH₃: Ammonia concentration; **TSCFA:** Total short-chain fatty acid.; CI (50% Cassava pulp + 50% *Indigofera zollingeriana* branch) silage as the basal feed; **CIIZM:** 70% basal feed + 30% IZM; **CIJBM:** 70% basal feed + 30% JBM; **CIPKM:** 70% basal feed + 30% PKM; **CISBM:** 70% basal feed + 30% SBM; **CIBSM:** 70% basal feed + 30% BSM; **SEM:** Standard error of the mean; ^{a-c}Means in the same row without a common letter are different at P < 0.05.

populations, promoting both fiber degradation and VFA production, essential for energy availability.

Ammonia concentrations reflect the fermentation efficiency of both protein and carbohydrate substrates, for enhancing microbial proliferation in the rumen. The observed ammonia levels across diets ranged (P < 0.05) from 7.05 mM (CI) to 12.35 mM (CIBSM and CIJBM mixture), with most values falling within the optimal range of 8 to 12 mM, as described by McDonald *et al.* (2022). Ammonia concentrations within this range ensure adequate nitrogen availability for microbial growth, which directly influences microbial protein synthesis, an essential process for ruminant growth and milk production (Dewhurst and Newbold 2022). The basal feed (CI) showed a slightly lower NH₃ concentration (7.05 mM) close to the threshold but might limit nitrogen availability for microbial protein synthesis. This could potentially affect animal performance, particularly in rapidly growing or high-producing ruminants that require a higher rate of microbial protein supply. Higher ammonia levels observed in the CIBSM and CIJBM mixture suggest enhanced protein degradation and nitrogen utilization, which could support improved growth rates and feed efficiency. Excessively high ammonia concentrations (>21 mM) are toxic, leading to inefficient nitrogen utilization and increased nitrogen excretion (Shen *et al.*, 2023). The observed ammonia levels show that none of the diets pose a risk of ammonia toxicity while still supporting microbial protein synthesis efficiently. Future studies could further explore the relationship between ammonia concentration and specific performance metrics, such as growth rates, milk yield, or nitrogen retention efficiency.

TSCFA serves as a key indicator of ruminal fermentation efficiency and provides a crucial energy source for ruminants. In this study, TSCFA concentrations ranged (P < 0.05) from 71.74 mM (basal silage) to 131.68 mM (CIBSM mixture). High TSCFA concentrations suggest enhanced microbial fermentation, which results in greater VFA production, providing energy for the ruminant. VFA including acetate, propionate, and butyrate are the primary sources of energy derived from feed fermentation in the rumen, with propionate functioning as a glucogenic precursor that supports growth and milk production (Van

Soest, 2023). The TSCFA values for the CIBSM mixture (131.68 mM) show robust fermentation efficiency, which may translate to higher energy production and improved animal performance.

The lower TSCFA concentration observed in the basal silage (71.74 mM) suggests suboptimal fermentation efficiency, potentially limiting energy availability. Although within the normal range, the lower TSCFA levels show that diets such as CIBSM, containing high levels of fermentable substrates, provide superior fermentation conditions. CIIZM and CIPKM mixture showed TSCFA concentrations ranging from 91.53 to 107.04 mM, supporting moderate fermentation efficiency. These results are consistent with Baek *et al.* (2022), who reported that typical TSCFA concentrations in tropical forages, such as Napier grass and Sorghum, range between 72.65 mM and 86.32 mM. Therefore, the inclusion of higher fermentable carbohydrate sources such as black soldier fly larvae meal may enhance overall energy yield and promote improved growth performance.

The interplay between rumen pH, ammonia concentration, and TSCFA production is driven by the balance between structural and non-structural carbohydrates. Diets high in non-structural carbohydrates such as starch and sugars enhance VFA production, particularly propionate, which competes with methanogenesis for hydrogen, reducing methane emissions (Pereira *et al.*, 2022). In contrast, diets rich in structural carbohydrates such as NDF, and ADF ferment more slowly, producing more acetate and butyrate, associated with higher methane production (Ridla *et al.*, 2023b). Optimizing the carbohydrate profile to favor rapid fermentation of non-structural carbohydrates can improve both energy efficiency and environmental sustainability by reducing methane emissions and enhancing microbial protein synthesis (Sun *et al.*, 2022).

IN VITRO GAS PRODUCTION, METHANE EMISSION AND DEGRADABILITY

Table 5 shows the total gas production, methane emission, as well as degradability characteristics of the CI and various mixture diets, indicating significant differences (P < 0.05) in fermentation activity, methane production, and degradability.

Table 5: Gas production, methane emission, and degradability of CI Silage and mixture diets.

Variable	CI Silage	CIIZM	CIJBM	CIPKM	CISBM	CIBSM	SEM	P-value
Gas, ml/g	141.92 ^d	158.05 ^b	157.95 ^b	153.80 ^c	159.40 ^a	159.17 ^a	2.63	0.032
Methane, %TSCFA	20.78 ^a	12.02 ^d	14.41 ^c	15.68 ^b	11.33 ^d	11.59 ^d	0.38	0.015
IVDMD, %	58.98 ^c	65.94 ^a	64.29 ^b	64.50 ^b	65.79 ^a	65.87 ^a	0.54	0.046
IVOMD, %	57.11 ^a	64.42 ^b	61.37 ^c	62.35 ^c	64.21 ^c	64.30 ^b	0.39	0.021

IVDMD: in vitro dry matter degradability; **IVOMD:** in vitro organic matter degradability; **CI** (50% Cassava pulp + 50% *Indigofera zollingeriana* branch) silage as the basal feed; **CIIZM:** 70% basal feed + 30% IZM; **CIJBM:** 70% basal feed + 30% JBM; **CIPKM:** 70% basal feed + 30% PKM; **CISBM:** 70% basal feed + 30% SBM; **CIBSM:** 70% basal feed + 30% BSM; **SEM:** Standard error of the mean; ^{a-d}Means in the same row without a common letter are different at $P < 0.05$.

Gas production is a key indicator of rumen fermentation, as it reflects microbial activity in feed degradability. Elevated gas production generally shows enhanced fermentation activity and more comprehensive nutrient degradation in the rumen. However, increased gas production can also be linked to undesirable byproducts, such as methane, a potent greenhouse gas. Balancing gas production with methane emissions is critical for optimizing nutrient utilization while minimizing environmental impact.

In the data, gas production varies significantly ($P < 0.05$) among the diets. The highest gas production was observed in CISBM at 159.40 ml/g DM, followed by CIBSM mixture at 159.17 ml/g DM. These results suggest that the diets effectively promote active fermentation and nutrient degradation. In contrast, CI showed the lowest gas production at 141.92 ml/g DM, suggesting reduced fermentative activity.

Although elevated gas production reflects enhanced rumen fermentation, it is often accompanied by increased methane emissions. Methane is primarily produced by methanogenic archaea as a byproduct of hydrogen utilization during fermentation. Diets that increase gas production without excessive methane generation are preferable, showing efficient fermentation. In this study, the high gas production in diets such as CISBM and CIBSM was associated with improved ammonia levels, indicative of efficient fermentation of both protein and carbohydrate sources, likely supporting microbial growth. This corresponds with enhanced TSCFA production, improving energy availability for the animal (Harris *et al.*, 2021; Kara *et al.*, 2022). However, balancing gas production with methane emissions remains crucial.

Methane production is a critical consideration when evaluating the environmental impact of ruminant diets. It accounts for 2-12% of total ingested energy, reflecting an efficiency loss in ruminants, which impacts both productivity and environmental footprint. Lower methane emissions are desirable, not only for environmental reasons but also to improve feed efficiency. Based on the results, CI produced the highest methane emissions, with methane constituting

20.78% of TSCFA. This suggests inefficient fermentation and greater energy losses, which can negatively affect both animal performance and environmental sustainability. In contrast, CIBSM, CIIZM, and CISBM mixture showed significantly lower methane emissions at 11.59%, 12.02%, and 11.33% of TSCFA, respectively. These results show that the diets promote more efficient fermentation, leading to reduced methane emissions and less energy loss. CIBSM and CIIZM diets reduce methane emissions, but are not superior to CISBM in this regard, suggesting that further optimization may be required to fully replace soybean meal in terms of methane reduction. The reduced methane emissions observed in the CIBSM and CIIZM diets can be attributed to the presence of highly digestible components that enhance fermentation efficiency, such as non-fibrous carbohydrates (NFC). Dong *et al.* (2022) reported that a higher NFC content accelerates fermentation and reduces hydrogen availability for methanogenesis, thereby lowering methane emissions. Additionally, the lower methane output from the diets suggests improved nutrient utilization, which may have positive implications for long-term animal productivity, including growth rates and feed efficiency.

Degradability, specifically IVDMD and IVOMD, shows how effectively an animal can degrade and use feed. Higher degradability reflects better nutrient absorption, improved animal performance, and reduced waste output. Moreover, greater degradability can contribute to lower methane emissions, as more efficient nutrient utilization in the rumen reduces fermentation byproducts including methane. In this study, the highest IVDMD was found in CIIZM at 65.94%, followed closely by CIBSM at 65.87% and CISBM at 65.79%. In contrast, CI showed the lowest IVDMD at 58.98%, suggesting less efficient digestion. IVOMD results also mirrored this trend, with CIIZM achieving the highest organic matter degradability at 64.42%, and CI showing the lowest value at 57.11%. These results suggest that the alternative protein sources (CIIZM and CIBSM) support efficient nutrient utilization comparable to soybean meal, offering viable options for sustainable ruminant feeding strategies.

The differences in degradability are primarily driven by the diets protein and fiber content. Diets higher in CP and lower in fiber tend to have greater degradability, supporting more efficient rumen fermentation and nutrient absorption (Samadi *et al.*, 2023). For instance, the inclusion of *Indigofera zollingeriana* and black soldier fly larvae meal, both rich in digestible proteins, enhances microbial activity, promoting higher IVDMD and IVOMD values. These results were consistent with Panneerselvam *et al.* (2024), where high-protein diets enhanced degradability and reduced methane emissions by limiting hydrogen availability for methanogens.

The relationship between these parameters is largely influenced by the balance of nutrients in the diet. Based on the results, diets rich in fermentable carbohydrates increase gas production, which can support microbial growth and enhance nutrient degradability. However, when not balanced, this may lead to elevated methane emissions. The diets examined in this study show that optimizing protein and carbohydrate sources, including the inclusion of *Indigofera zollingeriana* leaf meal and black soldier fly larvae meal, can enhance fermentation efficiency as showed by higher gas production and degradability while minimizing methane emissions. This balance is crucial for improving the energy efficiency of feed, which directly impacts animal productivity and environmental sustainability.

The long-term benefits of diets that reduce methane emissions and improve nutrient degradability extend beyond immediate fermentation efficiency. Diets such as CIIZM and CIBSM could potentially enhance growth rates, improve reproductive performance, and reduce health challenges associated with inefficient digestion, including bloating and acidosis. Moreover, the reduction in methane emissions is consistent with global efforts to mitigate climate change, making these diets more sustainable for large-scale livestock production. Strategies such as supplementing feed with tannins, methane inhibitors, or manipulating the rumen microbiome can further enhance methane-reducing potential of the diets (Cristobal-Carballo, 2021; Doan *et al.*, 2023). In summary, while the CIBSM and CIIZM diets show promise in reducing methane emissions and improving degradability, further studies are needed to assess long-term impacts on ruminant health and productivity. Incorporating these alternative protein sources into practical feeding regimes may contribute to more sustainable livestock production, particularly in terms of environmental impact and energy efficiency.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this study suggested that black soldier fly larvae meal and *Indigofera zollingeriana* leaf meal could

serve as effective alternatives to conventional soybean meal in livestock diets. These feed components improved fermentation efficiency, reduced methane emissions, and enhanced nutrient degradability, offering viable options for promoting both livestock productivity and environmental sustainability. By optimizing rumen fermentation and decreasing greenhouse gas emissions, these alternative protein sources can contribute to more sustainable livestock management practices.

Further investigation is needed to elucidate the long-term impacts of alternative diets on animal health, growth performance, and reproductive metrics. Additionally, empirical trials are necessary to evaluate the scalability and economic viability of integrating black soldier fly larvae meal and *Indigofera zollingeriana* leaf meal within diverse livestock production systems. Stakeholders should prioritize these sustainable feed sources to mitigate the environmental impact of ruminant agriculture while sustaining optimal productivity.

ACKNOWLEDGEMENTS

We sincerely thank the Feed Science and Technology Laboratory staff, Faculty of Animal Science, IPB University, for their support during data analysis. We also appreciate their assistance and access to facilities essential for this work.

NOVELTY STATEMENTS

This study introduced the integration of black soldier fly larvae meal and *Indigofera zollingeriana* leaf meal as alternative protein sources in livestock diets, underscoring the potential to reduce methane emissions and improve rumen fermentation efficiency, a combination rarely explored in previous studies. By using black soldier fly larvae meal, this study bridged the gap between sustainable insect-based feeds and conventional livestock nutrition, presenting a novel solution for addressing both nutritional demands and environmental sustainability in ruminant production.

The use of *Indigofera zollingeriana* leaf meal shows the dual role in enhancing nutrient degradability and lowering greenhouse gas emissions, marking it as a valuable, eco-friendly forage alternative to traditional high-protein feedstocks including soybean meal. This study contributes to the field of sustainable livestock farming by offering new insights into alternative feed sources that can reduce the reliance on soy-based diets, in line with global efforts to promote resource efficiency and mitigate climate change impacts from agriculture.

AUTHOR'S CONTRIBUTIONS

Both authors contributed to designing and implementing

the study, analyzing the results, and drafting the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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